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GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

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I. SCOPE OF THE GUIDELINES

I-A. *Purpose.* The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

I-B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the replication of those described in (i) above.

I-C. *General Applicability.* See Section IV-B.

I-D. *Prohibitions.* The following experiments are not to be initiated at the present time:

I-D-1. Formation of recombinant DNAs derived from the pathogenic organisms classified[1] as Class 3, 4, or 5[2] or from cells known [2A] to be infected with such agents, regardless of the host-vector system used.

I-D-2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates [2A] (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.).

I-D-3. Deliberate creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange. [2A]

I-D-4. Deliberate release into the environment of any organism containing recombinant DNA.

I-D-5. Deliberate transfer of a drug resistence trait to microorgansims that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. [2A]

I-D-6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established [3]. (See Section IV-E-1-b-(3)-(d).)

We differentiate between small- and large-scale experiments with organisms containing recombinant DNAs because the probability of escape from containment barriers normally increases with increasing scale.

Experiments in these categories may be excepted[4] from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly approved by the Director, NIH, with advice of the Recombinant DNA Advisory Committee after appropriate notice and opportunity for public comment. (Section IV-E-1-b-(1)-(e).)

I-E. *Exemptions.* It must be emphasized that the following exemptions[4] are not meant to apply to experiments described in the Section I-D-1 to I-D-5 as being prohibited.

The following recombinant DNA molecules are exempt from these Guidelines, and no registration with NIH is necessary:

I-E-1. Those that are not in organisms or viruses.[5]

I-E-2. Those that consist entirely or DNA segments from a single nonchromosomal or viral DNA source, though

one or more of the segments may be a synthetic equivalent.

I-E-3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or closely related strain of the same species) or when transferred to another host by well-established physiological means; also those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

I-E-4. Certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment. (See Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20014.

I-E-5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV-E-1-b-(1)-(d).)

I-F. *General Definitions.* See Section IV-C.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. [6-19] The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) A set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs, by their very nature, lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a *vector*, or *vehicle*, (plasmid or virus) for specific hosts or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the Guidelines.

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and institutional biosafety committees recommend changes in the Guidelines to permit their use.

II-A. *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. [6-15] Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. (see Sections IV-D-1-g, IV-D-5-d and IV-D-8-b.). This shall as a minimum include instructions in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. (See Sections IV-D-5-e and IV-D-3-d.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provided. (See Sections IV-D-1-h and IV-D-8-c.)

II-B. *Physical Containment Levels.* The objective of physical containment is to confine organisms containing recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. For example, the "Classification of Etiologic Agents on the Basis of Hazard," [7] prepared by the Center for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels. [8]

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV-E-1-b-(2)-(b).) Additional material on physical containment for plant host-vector systems is found in Sections III-C-3 and III-C-4.

II-B-1. *P1 Level.*

II-B-1-a. *Laboratory Practices.*

II-B-1-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-1-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II-B-1-a-(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decontaminated before washing, reuse, or disposal.

II-B-1-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-1-a-(5). Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-1-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-1-a-(7). Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

II-B-1-a-(8). Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-1-a-(9). An insect an rodent control program shall be instituted.

II-B-1-a-(10). The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

II-B-1-a-(11). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-1-a-(12). The laboratory shall be kept neat and clean.

II-B-1-b. *Containment Equipment.* Special containment equipment is not required at the P1 level.

II-B-1-c. *Special Laboratory Design.* Special laboratory design is not required at the P1 level.

II-B-2. *P2 Level.*

II-B-2-a. *Laboratory Practices.*

II-B-2-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-2-a-(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombitant DNA molecules.

II-B-2-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

II-B-2-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-2-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-2-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-2-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-2-a-(8). Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-2-a-(9). Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

II-B-2-a-(10). The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-2-a-(11). An insect and rodent control program shall be instituted.

II-B-2-a-(12). The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II-B-2-a-(13). Animals not related to the experiment shall not be permitted in the laboratory.

II-B-2-a-(14). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-2-a-(15). The laboratory shall be kept neat and clean.

II-B-2-a-(16). Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II-B-2-b. *Containment Equipment.* Biological safety cabinets[20] shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

II-B-2-c. *Special Laboratory Design.* An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

II-B-3. *P3 Level.*

II-B-3-a. *Laboratory Practices.*

II-B-3-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-3-a-(2). Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms contining recombinant DNA molecules.

II-B-3-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II-B-3-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-3-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II-B-3-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-3-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-3-a-(8). Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II-B-3-a-(9). Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

II-B-3-a-(10). Persons under 16 years of age shall not enter the laboratory.

II-B-3-a-(11). The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II-B-3-a-(12). An insect and rodent control program shall be instituted.

II-B-3-a-(13). Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

II-B-3-a-(14). Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

II-B-3-a-(15). Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

II-B-3-a-(16). Animals and plants not related to the experiment shall not be permitted in the laboratory.

II-B-3-a-(17). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II-B-3-a-(18). Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

II-B-3-a-(19). The laboratory shall be kept neat and clean.

II-B-3-a-(20). If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

II-B-3-b. *Containment Equipment.*

II-B-3-b-(1). Biological safety cabinets [20] shall be used for all equipment and manipulations that produce aerosols—e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging—where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

II-B-3-b-(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units [19A], open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolt radiation lamps and reflectors. (Note: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

II-B-3-b-(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

TABLE I.—*Combinations of Containment Safeguards*

Classification of experiment according to Guidelines		Alternate combinations of physical and biological containment			
		Physical Containment			
Physical containment	Biological* containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P3	HV3	P3	P3	P3	HV3
P3	HV3	P3	P3	P4	HV2
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P2	HV3
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P3	P2	HV2

*See section II-D for description of biological containment.

II-B-3-c. *Special Laboratory Design.*

II-B-3-c-(1). The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

II-B-3-c-(2). The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

II-B-3-c-(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near each primary laboratory exit area.

II-B-3-c-(4). Windows in the laboratory shall be sealed.

II-B-3-c-(5). An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II-B-3-c-(6). The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential (i.e. from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhause air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

II-B-3-c-(7). The treated exhaust-air from Class I and Class II biological safety cabinets [20] may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air

balance of the cabinet and the building ventilation system.

II-B-4. *P4 Level.*

II-B-4-a. *Laboratory Practices.*

II-B-4-a-(1). Laboratory doors shall be kept closed while experiments are in progress.

II-B-4-a-(2). Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

II-B-4-a-(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II-B-4-a-(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II-B-4-a-(5). Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

II-B-4-a-(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II-B-4-a-(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II-B-4-a-(8). Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

II-B-4-a-(9). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

II-B-4-a-(10). Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

II-B-4-a-(11). Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

II-B-4-a-(12). Persons under 18 years of age shall not enter the P4 facility.

II-B-4-a-(13). Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

II-B-4-a-(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

II-B-4-a-(15). The universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

II-B-4-a-(16). An insect and rodent control program shall be instituted.

II-B-4-a-(17). Animals and plants not related to the experiment shall not be permitted in the laboratory in which the experiment is being conducted.

II-B-4-a-(18). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II-B-4-a-(19). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II-B-4-a-(20). The laboratory shall be kept neat and clean.

II-B-4-a-(21). If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4-level containment, they shall be conducted in accordance with all P4-level laboratory practices specified in this section.

II-B-4-b. *Containment Equipment.*

II-B-4-b-(1). Experimental procedures involving organisms that require P4-level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure isolation suits.

II-B-4-b-(2). Laboratory animals involved in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial-containment caging systems (such as Horsfall units [19A], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure suits.

II-B-4-b-(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II.

TABLE II.—*Combinations of Containment Safeguards*

Classification of experiment according to Guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological * containment	Laboratory design specified for:	Laboratory practices specified for:	Containment equipment specified for:	Biological containment
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	P4 **	P3	HV2

* See Section II-D for description of biological containment.** In this case gloves shall be worn, in addition to the clothing requirements specified in II-B-4-a-(14).

II-B-4-c. *Special Laboratory Design.*

II-B-4-c-(1). The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

II-B-4-c-(2). Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces are sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

II-B-4-c-(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

II-B-4-c-(4). Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply and liquid and gaseous servies provided to the P4 facility shall be protected by devices that prevent backflow.

II-B-4-c-(5). Drinking water fountains shall not be installed in laboratory or animal rooms of the P4 facility. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

II-B-4-c-(6). Laboratory doors shall be self-closing.

II-B-4-c-(7). A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

II-B-4-c-(8). A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

II-B-4-c-(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be inactivated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

II-B-4-c-(10). An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

II-B-4-c-(11). Air within individual laboratories of the P4 facility may be recirculated if HEPA filtered.

II-B-4-c-(12). The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow *in situ* decontamination before removal and to facilitate certification testing after replacement.

II-B-4-c-(13). The treated exhaust-air from Class I and Class II bioligical safety cabinets [20] may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets or the facility exhaust air system.

II-B-4-b-(14). As noted in Section II-B-4-c-(1), the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be airtight. The exhaust-air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support sysem shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

II-C. *Shipment.* Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service and the U.S. Department of Transportation (§ 72.25, Part 72, Title 42, and §§ 173.386-.388, Part 173, Title 49, U.S. Code of Federal Regulations) as specified below:

II-C-1. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of § 72.25(c)(1)-(5), Part 72, Title 42 CFR, and §§ 173.386-.388, Part 173, Title 49 CFR.

II-C-2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offerd for transportation or transported, are subject to the requirements listed above under II-C-1 and are also subject to § 72.25(c)(6), Part 72, Title 42 CFR.

II-C-3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Reombinant DNA Research."

II-D. *Biological Containment.*

II-D-1. *Levels of Biological Containment.* In consideration of biological containment, the vector (Plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) Survival of the vector in its host outside the laboratory and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

II-D-1-a. *HV1.* A host-vector system which provides a moderate level of containment. *Specific systems:*

II-D-1-a-(1). *EK1.* The host is always *E. Coli* K-12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1, or derivatives thereof [21-27]) and variants of bacteriophage, such as [28-33]. The *E. Coli* K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

II-D-1-a-(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. Col.* K-12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and mechanism for approval of such HV1 systems are described below (Section II-D-2).

II-D-1-b. *HV2* These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. *Specific systems:*

II-D-1-b-(1). For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

II-D-1-b-(2). For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

II-D-1-c. *HV3.* These are host-vector systems in which:

II-D-1-c-(1). All HV2 criteria are met.

II-D-1-c-(2). The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

II-D-1-c-(3). No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or on unique laboratory-controlled conditions or is blocked by the inserted DNA.

II-D-1-c-(4). The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

II-D-1-c-(5). The relevant genotypic and phenotypic traits have been independently confirmed.

II-D-2. *Certification of Host-Vector Systems.*

II-D-2-a. *Responsibility.* HV1 systems other than *E. coli* K-12, and HV2 and HV3 host-vector systems, may not be designated as such until they have been certified by the Director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20014.

Host-vector systems that are proposed for certification will be reviewed by the NIH Recombinant DNA Advisory Committee (RAC). (See Section IV-E-1-b-(1)-(c).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available information at a regular meeting. The Director, NIH is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH without review by the RAC. (See Section IV-E-1-b-(3)-(h).)

When new host-vector systems are certified, notice of the certification will be sent by ORDA to the applicant and to all IBCs and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH may at any time rescind the certification of any host-vector system. (See Section IV-E-1-b-(3)-(i).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the Director, NIH. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

II-D-2-b. *Data To Be Submitted for Certification.*

II-D-2-b-(1). *HV1 Systems Other than E. Coli K-12.* The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

II-D-2-b-(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted [33A, 33B]. In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the NIH Recombinant DNA Advisory Committee (RAC). Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More spe-

cific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage ● in *E. coli* K-12 are available from ORDA.

II-D-2-b-(3). *HV3 Systems.* Putative HV3 systems must, as the first step in certification, be certified as HV2 systems. Systems which meet the criteria given above under II-D-1-(c)-1, II-D-1-(c)-2, II-D-1-(c)-3 will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selected by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated. Tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

II-D-3. *Distribution of Certified Host-Vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates. If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotyic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH; and (iv) remind the user of responsibility for notifying ORDA of any discrepancies with the reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Containment guidelines for permissible experiments are given in Part III. Changes in these levels for specific experiments (or the assignment of levels to experiments not explicitly considered here) may not be instituted without the express approval of the Director, NIH. (See Sections IV-E-1-b-(1)-(a), IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(b), IV-E-1-b-(2)-(c), and IV-E-1-b-(3)-(b).)

III-A. *Classification of Experiments Using the E. coli K-12 Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K-12 host-vector systems. These are the systems for which we have the most experience and knowledge (i) regarding the effectiveness of biological containment provided by existing hosts and vectors and (ii) necessary for the construction of more effective biological barriers. We therefore consider DNA recombinants in *E. coli* K-12 before proceeding to other host-vector systems. The levels of biological containment for *E. coli* K-12 systems are designated EK1, EK2, and EK3 in ascending order.

It has been necessary, throughout this section, to use words and such terms are marked with footnote reference numbers. These footnotes (Part V) define more fully what the terms denote.

In the following classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimal for the experiments designated. The use of higher levels of biological containment (EK3>EK2>EK1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

III-A-1. *Shotgun Experiments.* These experiments involve the production of recombinant DNAs between the vector and portions of the specified cellular source, preferably a partially purified fraction. Care should be taken either to preclude or eliminate contaminating microorganisms before isolating the DNA.

III-A-1-a. *Eukaryotic DNA Recombinants.*

III-A-1-a-(1). *Primates.* P2 physical containment+an EK2 host-vector. Any lowering of containment below these levels (i.e., for purified DNA or characterized clones) cannot be made solely by an institutional biosafety committee but requires NIH approval. (See Section IV-E-1-b-(3)-(e).)

III-A-1-a-(2). *Other Mammals.* P2 physical containment+an EK2 host-vector.

III-A-1-a0(3). *Birds.* P2 physical containment+an EK2 host-vector, or P3+EK1.

III-A-1-a-(4). *Cold-Blooded Vertebrates.* P2 physical containment+an EK1 host-vector or P1+EK2. If the eukaryote is known to produce a potent polypeptide toxin, [34] the containment shall be increased to P3+EK2.

III-A-1-a-(5). *Other Cold-Blooded Animals and Lower Eukaryotes.* This large class of eukaryotes is divided into two groups:

III-A-1-a-(5)-(a). Species that are known to produce a potent polypeptide toxin [34] that acts in vertebrates, or are known pathogens listed in Class 2, [1] or are known to carry such pathogens must use P3 physical containment+an EK2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3+EK1 or P2+EK2. Species that produce potent toxins that affect invertebrates or plants but not vertebrates require P2+EK2 or P3+EK1. Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group, unless the organisms used as the source of DNA have been shown not to contain those agents, in which case they may be placed in the following group. [2A]

III-A-1-a-(5)-(b). The remainder of the species in this class including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2 + EK1 or P1 + EK2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of disease-causing microorganisms or must belong to a species that does not carry microorganisms causing disease in vertebrates or plants. [2A] If these conditions cannot be met, experiments must be done under P3 + EK1 or P2 + EK2 containment.

III-A-1-a-(6). *Plants.* P2 physical containment + an EK1 host-vector, or P1 + EK2. If the plant source makes a potent polypeptide toxin, [34] the containment must be raised to P3 physical containment + an EK2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked plant genes, containment may be reduced to P3 + EK1 or P2 + EK2. [2A]

III-A-1-b. *Prokaryotic DNA Recombinants.*

III-A-1-b-(1). *Prokaryotes That Exchange Genetic Information [35] with E. Coli.* Those prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I-E-4).

For those not on the list, the containment levels are P1 physical containment + an EK1 host-vector. In

fact, experiments in this category may be performed with any *E. coli* K-12 vector (e.g., conjugative plasmids). However, for prokaryotes that are classified [1] as Class 2, the containment levels are P2 + EK1.

III-A-1-b-(2). *Prokaryotes That Do Not Exchange Genetic Information with E. Coli.* P2 physical containment + an EK1 host-vector, or P1 + EK2, except for DNA from Class 2 agents, [1] which require P3 + EK2.

III-A-2. *Plasmids, Bacteriophages, and Other Viruses.* Recombinants formed between a vector and some other plasmid or virus DNA have in common the potential for acting as double vectors because of the replication functions in these DNAs. The containment conditions given below apply only to propagation of the DNA recombinants in *E. coli* K-12 hosts. They do not apply to other hosts in which the recombinants may be able to replicate as a result of functions provided by the DNA inserted into the EK vectors. These are considered under other host-vector systems.

III-A-2-a. *Viruses of Eukaryotes* (summary given in Table III).

III-A-2-a-(1). *DNA Viruses.*

III-A-2-a-(1)-(a). *Nontransforming viruses.*

III-A-2-a-(1)-(a)-(*1*). *Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (Strain FL), and Plant Viruses.* P1 physical containment + and EK1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA. [37]

III-A-2-a-(1)-(a)-(*2*). *Hepatitis B.*

III-A-2-a-(1)-(a)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for purified subgenomic DNA segments. [38]

III-A-2-a-(1)-(a)-(*2*)-(*b*). P2 physical containment + an EK2 host-vector, or P3 + EK1, shall be used for DNA for recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(a)-(*2*)-(*c*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA. [37]

III-A-2-a-(1)-(a)-(*3*). *Other Nontransforming Members of Presently Classified Viral Families.* [*36*]

III-A-2-a-(1)-(a)-(*3*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA[38] segments or (ii) purified cDNA copies of viral mRNA. [37]

III-A-2-a-(1)-(a)-(*3*)-(*b*). P1 physical containment + an EK1 host and a vector certified for use in an EK2 system shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(b). *Transforming Viruses.* [37A]

III-A-2-a-(1)-(b)-(*1*). *Herpes Saimiri, Herpes Ateles, and Epstein Barr Virus.* [*39*]

III-A-2-a-(1)-(b)-(*1*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments. [38]

III-A-2-a-(1)-(b)-(*1*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for (i) DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene [38] or (ii) purified cDNA copies of viral mRNA. [37]

III-A-2-a-(1)-(b)-(*1*)-(*c*). P3 physical containment + an EK1 host-vector, or P2 + EK2, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(1)-(b)-(*2*). *Other Transforming Members of Presently Classified Viral Families.* [*36*]

III-A-2-a-(1)-(b)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments. [38]

III-a-2-a-(1)-(b)-(*2*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) subgenomic DNA segments containing an entire transforming gene, (iii) purified cDNA copies of viral mRNA, [37] or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(2). *DNA Transcripts of RNA Viruses.*

III-A-2-a-(2)-(a). *Retroviruses.*

III-A-2-a-(2)-(a)-(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses.* [*39*]

III-A-2-a-(2)-(a)-(*1*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments. [38]

III-A-2-a-(2)-(a)-(*1*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for DNA recombinants produced with purified subgenomic DNA segments [38] containing an entire transforming gene.

III-A-2-a-(2)-(a)-(*1*)-(*c*). P2 physical containment + an EK2 host-vector, or P3 + EK1, shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) purified cDNA copies of viral mRNA, [37] or (iii) subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(2)-(a)-(*2*). *Other Members of the Family Retroviridiae.* [36]

III-A-2-a-(2)-(a)-(*2*)-(*a*). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments. [38]

III-A-2-a-(2)-(a)-(*2*)-(*b*). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for DNA recombinants produced with (i) subgenomic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA, [37] or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III-A-2-a-(2)-(b). *Negative Strand RNA Viruses.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. [37]

III-A-2-a-(2)-(c). *Plus-Strand RNA Viruses.*

III-A-2-a-(2)-(c)-(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + and EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. [37]

III-A-2-a-(2)-(c)-(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families.* [*36*]

Table III

Recommended Containment for Cloning of Viral DNA or cDNA in E. coli K-12 Host-Vector Systems
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
DNA					
Nontransforming viruses					
AAV, MVM, Mouse Adeno (Strain FL)	P1 + EK1		P1 + EK1		P1 + EK1
Plant Viruses	P1 + EK1		P1 + EK1		P1 + EK1
Hepatitis B	P1 + EK1[38]		P2 + EK2 or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Other	P1 + EK1[38]		P1 + EK1CV[40]		P1 + EK1
Transforming Viruses					
Herpes Saimiri, H. Ateles and EBV[39]	P1 + EK1[38]	P2 + EK1CV[40] or P3 + EK1[38]	P2 + EK2 or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Other	P1 + EK1[38]	P2 + EK1CV[40] or P3 + EK1	P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
RNA					
Retroviruses					
Gibbon Ape, Woolly Monkey FeLV and FeSV[39]	P1 + EK1[38]	P2 + EK1CV[40] or P3 + EK1[38]	P2 + EK2 or P3 + EK1		P2 + EK2 or P3 + EK1
Other	P1 + EK1[38]	P2 + EK1CV[40] or P3 + EK1	P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1

Table III

Recommended Containment for Cloning of Viral DNA or cDNA in E. coli K-12 Host-Vector Systems
(See text for full details)

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic[38]		Genomic		cDNA from viral mRNA[37]
	Nontransforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
Negative-Strand RNA	P1 + EK1		P1 + EK1	P1 + EK1	P1 + EK1
Plus-Strand RNA					
Types 1 and 2 Sabin Polio, 17D Yellow Fever Vaccine Strains	P1 + EK1		P1 + EK1		P1 + EK1
Other	P1 + EK1[38]		P2 + EK1CV[40] or P3 + EK1		P2 + EK1CV[40] or P3 + EK1
Double-Stranded RNA	P1 + EK1			P1 + EK1	P1 + EK1
Plant Viruses + Viroids	P1 + EK1		P1 + EK1	P1 + EK1	P1 + EK1
Intracellular Viral DNA	See text	See text	See text		

II-A-2-a-(2)-(c)-(2)-(a). P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments.[38]

III-A-2-a-(2)-(c)-(2)-(b). P2 physical containment + an EK1 host and a vector certified for use in an EK2 system, or P3 + EK1, shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, or (ii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(2)-(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(2)-(e). *RNA Plant Viruses and Plant Viroids.* P1 physical containment + an EK1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.[37]

III-A-2-a-(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see Section III-A-(1)-(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III-A-2-b. *Eukaryotic Organelle DNAs.* P2 physical containment + an EK1 host-vector, or P1 + EK2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III-A-2-c. *Prokaryotic Plasmid and Phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages.

III-A-3. *Lowering of Containment Levels for characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in case where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone made from a random assortment of DNAs has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III-A-3-a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified [41] and in which the absence of harmful sequences had been established [3] can be carried out under lower containment conditions than used for the corresponding shotgun experiment.[42] The containment may be decreased one step in physical containment (P4→P3; P3→P2; P2→P1) while maintianing the biological containment specified for the shotgun experiment, or one step in biological containment (EK3 → EK2; EK2 → EK1) while maintaining the specified physical containment. The institutional biosafety committee (IBC) must review such a reduction and the approval of the IBC must be secured before such a reduction may be put into effect. (See Section IV-D-3-b.) The IBC must notify the NIH Office of Recombinant DNA activities (ORDA) in writing of all such approvals within 30 days after they take place. IBC approval is sufficient for such a reduction except for (i) primate DNA, which also requires prior NIH approval (see Section III-A-1-a-(1)), or (ii) any lowering of containment under Section III-A-3-a to levels below P1 + EK1, which also requires prior NIH approval. (See Sections IV-D-1-c, IV-E-1-b-(3)-(e), and IV-E-1-b-(3)-(f).)

III-A-3-b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (3), experiments involving this recombinant DNA may be carried out under lower containment conditions, as described below.

III-A-3-b-(1). Institutional biosafety committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. (See Section IV-D-3-b.) The IBC must notify ORDA in writing of all such approvals within 30 days after they take place. IBC approval is sufficient for such a reduction except for (i) primate DNA, which requires prior NIH approval (see Section III-A-1-a-(1)), or (ii) any lowering of containment under Section III-A-3-b to levels below P1 + EK1, which also requires prior NIH Approval. (See Sections IV-D-1-c, IV-E-1-b-3-(e) and IV-E-1-b-(3)-(f).)

III-A-3-b-(2). Reduction of containment levels by more than one step, or cases involving primate DNA, or cases involving lowering of containment under Section III-A-3-b to levels below P1 + EK1, will require prior approval by NIH. (See Sections IV-E-1-b-(3)-(e), –(f) and –(g).)

III-B. *Experiments with Other Prokaryotic Host-Vectors.*

III-B-1. *HV1 Systems.* Host-vector systems which have been approved as HV1 systems may be used under P2 containment conditions for shotgun experiments with phages, plasmids, and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins.[34]

Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. Experiments with DNAs from eukaryotes (and their plasmids or viruses) will generally follow the criteria for the corresponding experiments with *E. coli* K-12 host-vectors if the major habitats of the given host-vector overlap those of *E. coli.* The habitats of other host-vector systems should also be considered in relation to containment.

II-B-2. *Return of DNA Segments to Non-HV1 Host of Origin.* Those prokaryotes tht exchange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I-E-4). For a prokaryote which can exchange genetic information [35] with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P-1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K-12 under P-1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a vector from a certified EK2 host-vector system and propagated in *E. coli* K-12 under the appropriate containment conditions [see Section III-A-1-b-(2)]. Subsequently, this recombinant DNA may be returned to Host B and propagated in Host B under P1 conditions.[43]

III-B-3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(c) and IV-E-1-b-(3)-(b).)

III-C. *Experiments with Eukaryotic Host-Vectors.*

III-C-1. *Vertebrate Host-Vector Systems.*[44] (Summary given in Table IV).

III-C-1-a. *Polyoma Virus.*

III-C-1-a-(1). *Productive Virus-Cell Interactions.*

III-C-1-a-(1)-(a). Defective or whole polyoma virus genomes, with appropri-

ate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III-C-1-a-(1)-(a)-(*1*). from bacteria of class 1 or class 2[1] or their phages or plasmids, except for those that produce potent polypeptide toxins; [34]

III-C-1-a-(1)-(a)-(*2*). from mice;

III-C-1-a-(1)-(a)-(*3*). from eukaryotic organisms that do not produce potent polypeptide toxins, [34] provided that the DNA segment is > 99% pure.

III-C-1-a-(1)-(b). Defective polyoma genomes, with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins. [34]

III-C-1-a-(1)-(c). Whole virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce poten polypeptide toxins. [34]

III-C-1-a-(1)-(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-a-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole polyoma virus genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-b. *Simian Virus 40.*

III-C-1-b-(1). *Productive Virus-Cell Interactions.*

III-C-1-b-(1)-(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-b-(1)-(a)-(*1*). bacteria of Class 1 or Class 2, -1] or their phages or plasmids, except for those that produce potent polypeptide toxins; [34]

III-C-1-b-(1)-(a)-(*2*). uninfected African green monkey kidney cell cultures.

III-C-1-b-(1)-(b). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins [34] (shotgun experiments or purified DNA).

III-C-1-b-(1)-(c). Experiments involving the use of defective SV40 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-b-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole SV40 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis -45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c. *Human Adenoviruses 2 and 5.*

III-C-1-c-(1). *Productive Virus-Cell Interactions.*

III-C-1-c-(1)-(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two essential genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III-C-1-c-(1)-(a)-(*1*). bacteria of Class 1 or Class 2[1] or their phages or plasmids except for those that produce potent polypeptide toxins; [34]

III-C-1-c-(1)-(a)-(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III-C-1-c-(1)-(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-c-(2). *Nonproductive virus-cell interactions.* Defective or whole human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis [45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d. *Murine Adenovirus Strain FL.*

III-C-1-d-(1). *Productive Virus-Cell Interactions.*

III-C-1-d-(1)-(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III-C-1-d-(1)-(a)-(*1*). bacteria of Class 1 or Class 2[1] or their phages or plasmids except for those that produce potent polypeptide toxins;[34]

III-C-1-d-(1)-(a)-(*2*). eukaryotic organisms that do not produce potent polypeptide toxins[34] (shotgun experiments or purified DNA).

III-C-1-d-(1)-(b). Experiments involving the use of whole murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d-(1)-(c). Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-d-(2). *Nonproductive Virus-Cell Interactions.* Defective or whole murine adenovirus strain FL genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-e. *All Other Potential Viral Vectors.*

III-C-1-e-(1). Experiments involving recombinant DNA molecules containing viral DNA segments consisting of 25% or less of the virus genome can be done:

III-C-1-e-(1)-(a). in P2 conditions when the recombinant DNA is to be integrated into the cell genome or is known to replicate as a plasmid in cells in culture, provided the additional DNA sequences are not derived from a eukaryotic virus. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis [45]

and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-1-e-(1)-(b). Under physical and biological containment conditions to be determined by NIH[45] when a viral helper will be used to propagate DNA sequences from prokaryotic or eukaryotic organisms. (See Section IV-E-1-b-(3)-(c).)

III-C-1-e-(2). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

NIH will also review on a case-by-case basis[45] all experiments involving the use of virus vectors in animals and will prescribe the physical and biological containment conditions appropriate for such studies. (See Section IV-E-1-b-(3)-(c).)

III-C-2. *Invertebrate Host-Vector Systems in Which Insect Viruses Are Used to Propagate Other DNA Segments.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, and integration of the viral DNA in vertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

III-C-3. *Plant Viral Host-Vector Systems.* The DNA plant viruses which could currently serve as vectors and cloning genes in plants and plant cell protoplasts are Cauliflower Mosaic Virus (CaMV) and its close relatives [2A] which have relaxed circular double-stranded DNA geromes with a molecular weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small (10^6 daltons) single-stranded DNA genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants shall be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-restrictive, preferably with positive air pressure, [2A] and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected materials shall be removed from the greenhouse or cabinet in sealed insect-proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary. Infected plant materials which have to be removed from the greenhouse or cabinet for further research shall be maintained under insect-restrictive conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The CaMV strain used as a cloning vector shall be a mutant that lacks the aphid transmission factor.

The viruses or their DNA may also be useful as vectors to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses provides adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is necessary, except as described in the following paragraph.

Experiments involving the use of plant virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis[45] and will be conducted under the prescribed physical and biological containment conditions. (See Section IV-E-1-b-(3)-(c).)

Table IV

Recommended Containment for Recombinant DNA Research Using Eukaryotic Viral Vectors
(See text for full details)

Vector DNA	Productive virus-cell interactions						Nonproductive virus-cell interactions[46]
	Type of DNA insert						
	Prokaryotic		Eukaryotic			Eukaryotic viral	
	Shotgun	Purified	Shotgun		Purified[47]		
			Natural host	Other			
1. Polyoma							
Intact Genome	P2	P2	P2	P3	P2	CBC*	P2
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2
2. SV40							
Intact Genome	—	—	—	—	—	—	P2
Deleted Genome	P2	P2	P2	P3	P3	CBC*	P2
3. Human Ad2+Ad5							
Deleted Genome	P3	P3	P3	P3	P3	CBC*	P2
4. Mouse Adenovirus (Strain FL)							
Intact Genome	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	P2
Deleted Genome	P2	P2	P2	P2	P2	CBC*	P2
5. Insect Viruses	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	—
6. Plant Viruses (CaMV and BGMV)	**	**	**	**	**	CBC*	—
7. All other potential Viral Vectors	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*	CBC*

*CBC = case-by-case[45]
**See text

III-C-4. *Plant Host-Vector Systems Other than Viruses.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (see Section I-E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit [2A] a decrease in the physical containment to P1.

Intact plants or propagative plant parts which cannot be grown in a standard P2 laboratory because of their large size may be grown under the P1 conditions described above in Section III-C-3, except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work.

III-C-5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.* The containment criteria for DNA recombinant experiments using these host-vectors most closely resemble those for prokaryotes, rather than those for the preceding eukaryotes, since the host cells usually exhibit a capacity for dissemination outside the laboratory that is similar to that for bacteria. Therefore, the procedures established for certification of HV systems other than *E. coli* K-12 (Section II-D-2) will also apply to these fungal or similar lower eukaryotic host-vector systems.

Once a HV1 system is approved by NIH, it may be used under P2 containment for shotgun experiments with phages, plasmids, and DNA from Class 1 prokaryotes[1] and lower eukaryotes that do not produce polypeptide toxins. [34] Other classes of recombinant DNA experiments with these HV1 systems will require prior approval and classification by NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b (2)-(c) and IV-E-1-b-(3)-(b).) If HV2 or HV3 systems of this type are developed and approved by NIH, guidelines for their use in other types of recombinant DNA experiments will also be established.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector from a certified EK2 host-vector system and propagated in *E. coli* K-12 under the appropriate containment conditions [see Section III-A-1-(a)-(5)]. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. [43]

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV-E-1-b-(1)-(b), IV-E-1-b-(2)-(c) and IV-E-1-b-(3)-(b).)

III-D. *Complementary DNAs.* Specific containment levels are given in Section III-A-a (see also last column of Table III) for complementary DNA (cDNA) or viral mRNA. For the other Sections of the Guidelines, where applicable, cDNAs synthesized *in vitro* are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under III-A-1, shotgun experiments; cDNAs formed from purified and characterized RNAs are included under III-A-3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III-E. *Synthetic DNAs.* If the synthetic DNA seqment is likely to [2A] yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product, [2A] it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment which corresponds to a nonharmful gene of birds, to be propagated in *E. coli* K-12, would require Ps physical containment plus an EK1 host-vector, or P1 + EK2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecules are exempt[4] from the Guidelines.

IV. Rules and Responsibilities

IV-A. *Policy.* Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgement are the key essentials to protection of health and the environment.

The Guidelines are intended to help the Institution, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the *purpose* of the Guidelines as well as to their specifics.

Each Institution (and the IBC acting on its behalf is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV-B. *General Applicability.* The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research performed by NIH directly.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an Institution that can and does assume the responsibilities assigned in these Guidelines.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

IV-C. *General Definitions.* The following terms, which are used throughout the Guidelines, are defined as follows:

IV-C-1. "DNA" means deoxyribonucleic acid.

IV-C-2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules which result from the replication of a molecule described in (i) above.

IV-C-3. "Memorandum of Understanding and Agreement" or "MUA" is a document that (i) provides to NIH or other Federal funding agency an Institution's certification that the recombinant DNA research project complies with the NIH Guidelines and (ii) contains other essential data as required

in the Administrative Practices Supplement.

IV-C-4. "Institution" means any public or private entity (including Federal, State, and local government agencies).

IV-C-5. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV-D-2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-D-2 and -3.

IV-C-6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV-E—3.

IV-C-7. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-E-2.

IV-C-8. "Director, NIH" or "Director" means the Director of the National Institutes of Health and any other officer or employee of NIH to whom authority has been delegated.

IV-C-9 "Federal Interagency Advisory Committee on Recombinant DNA Research" means the committee established in October 1976 to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH, on the coordination of those aspects of all Federal programs and activities which relate to recombinant DNA research.

IV-C-10. "Administrative Practice Supplement" or "APS" means a publication to accompany the NIH Guidelines specifying administrative procedures for use at NIH and at Institutions.

IV-C-11. "Laboratory Safety Monograph" or "LSM" means a publication to accompany the NIH Guidelines describing practices, equipment, and facilities in detail.

IV-D. *Responsibilities of the Institution*

IV-D-1. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV-D-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution, as part of its general responsibilities for implementing the Guidelines, may establish additional procedures, as deemed necessary, to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include (i) statements formulated by the Institution for general implementation of the Guidelines and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV-D-1-b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV-D-2 and carries out the functions detailed in Section IV-D-3.

IV-D-1-c. Submit, for each recombinant DNA project that meets with its approval, a Memorandum of Understanding and Agreement (MUA) to the funding agency for approval and registration. All projects, however, can proceed upon IBC approval (before submission of the MUA to the funding agency) except for the following, which require prior approval by NIH (or other funding agency designated by NIH for this purpose):

IV-D-1-c-(1). Projects for which containment levels are not specified by the Guidelines or NIH,

IV-D-1-c-(2). Projects requiring P4 containment,

IV-D-1-c-(3). Reductions of more than one step in containment levels (see Section III-A-3),

IV-D-1-c-(4). Reductions of containment level for projects involving primate DNA (see Section III-A-3),

IV-D-1-c-(5). Reductions of containment to levels below P1 + EK1 (see Section III-A-3),

IV-D-1-c-(6). The first project conducted in a facility at P3 containment, or

IV-D-1-c-(7). The first project conducted by an Institution.

NOTE: The MUA shall be submitted to the funding agency within 30 days of the IBC approval. If the funding agency does not routinely register recombinant DNA projects with NIH, the MUA must be submitted to NIH as well as to the funding agency. Authority to submit MUAs (or addenda) for which prior approval is not required may be delegated to the IBC chairperson. All MUAs that require NIH approval before the work can proceed shall be submitted to the NIH by the institutional official to whom the IBC is responsible.

IV-D-1-d. Take appropriate action to bring protocols into compliance when advised by NIH or other funding agency that IBC-approved projects do not conform to standards set forth in the Guidelines. This responsibility may be delegated to the IBC. (See Administrative Practices Supplement for further details).

IV-D-1-e. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section IV-D-4.

IV-D-1-f. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV-D-5, and assist investigators to do so.

IV-D-1-g. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC.

IV-D-1-h. Determine the necessity, in connection with each project, for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The Laboratory Safety Monograph (LSM) discusses various possible components of such a program—for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness.]

IV-D-1-i. Report within 30 days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV-D-2. *Membership and Procedure of the IBC.* The Institution shall establish an Institutional Biosafety Committee (IBC) meeting the following requirements:

IV-D-2-a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any potential risk to public health or the environment. At least two members (but not less than 20 percent of the mem-

bership of the committee) shall not be affiliated with the Institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community. The Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member (see Section IV-D-4).

IV-D-2-b. In order to ensure the professional competence necessary to review recombinant DNA activities, it is recomended that (i) the IBC include persons from disciplines relevant to recombinant DNA technology, biological safety, and engineering; (ii) the IBC include, or have available as consultants, persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be a nondoctoral person from a laboratory technical staff.

IV-D-2-c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require. (See the Administrative Practices Supplement for further guidance.)

IV-D-2-d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

IV-D-2-e. The Institution may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in section IV-D-3-a.)

IV-D-2-f. Central to implementation of the Guidelines is the review of proposed experiments by the IBC. The Institution shall submit, within 30 days of IBC approval, an MUA to NIH (ORDA), or shall otherwise register proposed experiments as specified under Sections IV-D-1-c, IV-D-1-d, and IV-F. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement.

IV-D-2-g. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection of privacy and proprietary interests.

IV-D-2-h. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public (e.g., MUAs, reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV-D-3. *Functions of the IBC.* On behalf of the Institution, the IBC is responsible for:

IV-D-3-a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research to be conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. (See Administrative Practices Supplement, II-D, for prior NIH approval requirements.) This review shall include:

IV-D-3-a-(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV-D-3-a-(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

NOTE: See Laboratory Safety Monograph (pages 187-190) for suggested guidance in conducting this review.

IV-D-3-b. Authorizing the Principal Investigator (PI) to proceed with the project upon receipt of proper agency approval; or authorizing the PI to proceed without agency approval to initiate or change a project for which none of the exceptions under IV-D-1-c apply.

NOTE: Some examples of work that might ordinarily proceed without prior funding-agency approval are the initiation of a project at the P1 or P2 level (other than the first project at the institution). Other examples are significant changes in hosts or vectors, in the donor species or the nature of the DNA segment selected, or in the physical location of the experiments. Still others are single-step reductions in containment level for (i) experiments with DNA recombinants from cellular DNAs that have been purified and are judged to be free of harmful sequences (see Section III-A-3-a) and for (ii) clones that have been characterized and judged to be free of harmful sequences (see Section III-A-3-b). It should be clear, however, that the funding agency must be notified of IBC approvals even when prior agency approval is not required. See the Administrative Practices Supplement for further discussion.

IV-D-3-c. Reviewing periodically recombinant DNA research being conducted at the Institution, to insure that the requirements of the Guidelines are being fulfilled.

IV-D-3-d. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

NOTE: Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph. Included are information and references on decontamination and emergency plans. NIH and the Center for Disease Control are available to provide consultation, and direct assistance if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant research-related illness or accident that appears to be a hazard to the public health.

IV-D-3-e. Reporting within 30 days to the appropriate institutional officials and to the NIH Office of Recombinant DNA Activities (ORDA) any signficant problems with or violations of the Guidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV-D-3-f. Performing such other functions as may be delegated to the IBC under Section IV-D-1.

IV-D-4. *Biological Safety Officer.* The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV-D-4-a. Insuring through periodic inspections that laboratory standards are rigorously followed;

IV-D-4-b. Reporting to the IBC and the Institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware, unless the BSO determines that the Principal Investigator (PI) has done so.

IV-D-4-c. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV-D-4-d. Providing advice on laboratory security;

IV-D-4-e. Providing technical advice to the PI and the IBC on research safety procedures.

NOTE: See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV-D-5. *Principal Investigator.* On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV-D-5-a. *PI—General.* As part of this general responsibility, the PI shall:

IV-D-5-a-(1). Initiate or modify no recombinant DNA research subject to the Guidelines until that research, or the proposed modification thereof, has been approved by the Institutional Biosafety Committee (IBC) and has met all other requirements of the Guidelines and the Administrative Practices Supplement (APS), and make changes to conform if the NIH Office of Recombinant DNA Activities' (ORDA's) review so requires;

IV-D-5-a-(2). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV-D-5-a-(3). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV-D-5-a-(4). Be adequately trained in good microbiological techniques;

IV-D-5-a-(5). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV-D-5-a-(6). Comply with shipping requirements for recombinant DNA molecules. (See Section II-C for shipping requirements, Laboratory Safety Monograph for technical recommendations, and the APS for administrative instructions and procedures. The requesting laboratory must be in compliance with the NIH Guidelines and under appropriate review by its IBC, and the sending investigator must maintain a record of all shipments of recombinant DNA materials.)

IV-D-5-b. *Submissions by the PI to NIH.* The PI shall:

IV-D-5-b-(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV-D-5-b-(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines (see Sections I-E-4 and I-E-5 and, for additional information on procedures, the APS); and

IV-D-5-b-(3). Petition NIH, with concurrence of the IBC, for exceptions to the prohibitions under these Guidelines (see Section I-D and, for additional information on procedures, the APS).

IV-D-5-c. *Submissions by the PI to the IBC.* The PI shall:

IV-D-5-c-(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV-D-5-c-(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV-D-5-c-(3). Submit the initial research protocol (and also subsequent changes—e.g., changes in the source of DNA or host-vector system, which require a new of revised Memorandum of Understanding and Agreement) to the IBC for review and approval or disapproval; and

IV-D-5-c-(4). Remain in communication with the IBC throughout the conduct of the project.

IV-D-5-d. *PI Responsibilities After Approval but Prior to Initiating the Research.* The PI is responsible for:

IV-D-5-d-(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV-D-5-d-(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV-D-5-d-(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV-D-5-e. *PI Responsibilities During the Conduct of the Approved Research.* The PI is responsible for:

IV-D-5-e-(1). Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

IV-D-5-e-(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV-D-5-e-(3). Correcting work errors conditions that may result in the release of recombinant DNA materials;

IV-D-5-e-(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, and genotypic and phenotypic characteristics); and

IV-D-5-e-(5). *Publications.* PIs are urged to include, in all publications reporting on recombinant DNA research, a description of the physical and biological containment procedures employed.

IV-E. *Responsibilities of NIH*

IV-E-1. *Director.* The Director, NIH, is responsible for (i) establishing the NIH Guidelines in recombinant DNA research, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has a number of responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV-E-1-a. *General Responsibilities of the Director, NIH.* The responsibilities of the Director shall include the following:

IV-E-1-a-(1). Promulgating requirements as necessary to implement the Guidelines;

IV-E-1-a-(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV-E-2. The RAC's membership is specified in its charter and in Section IV-E-2.

IV-E-1-a-(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV-E-3; and

IV-E-1-a-(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW, for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC.

IV-E-1-b. *Specific Responsibilities of the Director, NIH.* In carrying out the responsibilities set forth in this Section, the Director shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV-E-1-b-(1). *The Director is responsible for the following major actions* (For these, the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the FEDERAL REGISTER at least 30 days before the meeting, and the Director will also publish the proposed actions in the FEDERAL REGISTER for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the FEDERAL REGISTER for 30 days of comment before final action is taken. The Director's final decision, along with response to the comments, will be published in the FEDERAL REGISTER and the *Recombinant DNA Technical Bulletin.* The RAC and IBC chairpersons will be notified of this decision):

IV-E-1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV-E-1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV-E-1-b-(1)-(c). Certifying new host-vector systems, with the exception of minor modifications of already certified systems [The standards and procedures for certification are described in Section II-D-2-a. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment. See the Administrative Prac-

tices Supplement (APS) for further information];

IV-E-1-b-(1)-(d). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment (see Sections I-E-4 and -5 and the APS for further information);

IV-E-1-b-(1)-(e). Permitting exceptions to the prohibited experiments in the Guidelines, in order, for example, to allow risk-assessment studies; and

IV-E-1-b-(1)-(f). Adopting other changes in the Guidelines.

IV-E-1-b-(2). *The Director is also responsible for the following lesser actions.* (For these, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin*):

IV-E-1-b-(2)-(a). Interpreting and determining containment levels, upon request by ORDA;

IV-E-1-b-(2)-(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV-E-1-b-(2)-(c). Assigning containment levels for experiments not explicitly considered in the Guidelines (see Section III); and

IV-E-1-b-(2)-(d). Designating certain class 2 agents as class 1 for the purpose of these Guidelines (see Footnote 1 and Appendix B).

IV-E-1-b-(3). *The Director is also responsible for the following actions* (The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin*):

IV-E-1-b-(3)-(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV-E-1-b-(3)-(b). Determining appropriate containment conditions for experiments according to case precedence developed under Section IV-E-1-b-(2)-(c).

IV-E-1-b-(3)-(c). Determining appropriate containment conditions upon case-by-case analysis of experiments explicitly considered in the Guidelines but for which no containment levels have been set (see Footnote 45 in Part V; Sections III-C-1-a through -e; and Sections III-C -2 and -3);

IV-E-1-b-(3)-(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I-D-6);

IV-E-1-b-(3)-(e). Lowering containment levels one step for characterized clones involving primate DNA or for experiments using purified primate DNA (see Sections III-A-3-a and -b, and Footnotes 3 and 41);

IV-E-1-b-(3)-(f). Lowering containment levels for experiments involving other characterized clones or purified DNA below P1 + EK1 (see Sections III-A-3-a and -b, and Footnotes 3 and 41);

IV-E-1-b-(3)-(g). Lowering containment levels for characterized clones or purified DNA beyond one step (see Sections III-A-3-a and -b, and Footnotes 3 and 41);

IV-E-1-b-(3)-(h). Approving minor modifications of already certified host-vector systems (The standards and procedures for such modifications are described in Section II-D-2-a); and

IV-E-1-b-(3)-(i). Decertifying already certified host-vector systems.

IV-E-1-b-(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV-E-1-b-(5). The Director, at the end of 36 months from the time these Guidelines are promulgated, will report on the Guidelines, their administration, and the potential risks and benefits of this research. In doing so, the Director will consult with the RAC and the Federal Interagency Committee. Public comment will be solicited on the draft report and taken into account in transmitting the final report to the Assistant Secretary for Health and the Secretary, HEW.

IV-E-2. *Recombinant Advisory Committee.* The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious dieseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities.

All meetings of the RAC will be announced in the FEDERAL REGISTER, including tentative agenda items, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV-E-1-b-(1) may be added to an agenda after it appears in the FEDERAL REGISTER.

IV-E-2-a. *The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV-E-1-b-(1) and -(2).*

IV-E-3. *The Office of Recombinant DNA Activities.* ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Biological Safety Committees, Principal Investigators, Federal agencies, State and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV-E-1-b-(3). In addition, ORDA shall be responsible for the following:

IV-E-3-a. Review and approval of Institutional Biosafety Committee (IBC) membership;

IV-E-3-b. Registration of recombinant DNA projects; and

IV-E-3-c. Review of Memoranda of Understanding and Agreement (MUAs), and approval of those that conform to the Guidelines. In so doing, ORDA shall:

IV-E-3-c-(1). Conduct an independent evaluation of the containment levels required for the research covered by these Guidelines;

IV-E-3-c-(2). Determine whether the physical and biological containment levels approved by the IBC are in accordance with the requirement of the Guidelines;

IV-E-3-c-(3). Notify Institutions and the IBC chairperson in a timely fashion when MUAs (including changes in ongoing projects) do not conform to the Guidelines, and inform them of corrective measures to be taken;

IV-E-3-c-(4). Publish in the FEDERAL REGISTER:

IV-E-c-(4)-(a). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agendas 30 days in advance, with publication of the Director's proposed decision for 30 days of public and Federal agency comment followed by a published response, on any action listed in Section IV-E-1-b-(1); and

IV-E-3-c-(4)-(b). Announcements of RAC meetings and agendas 30 days in advance on any action listed in Section IV-E-1-b-(2).

NOTE.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV-E-3-c-(5). Publish the *Recombinant DNA Technical Bulletin*; and

IV-E-3-c-(6). Serve as executive secretary to the RAC.

IV-E-4. *Other NIH Components.* Other NIH components shall be responsible for:

IV-E-4-a. Awarding no grant or contract involving recombinant DNA techniques unless a properly executed MUA has been received;

IV-E-4-b. Certifying P4 facilities, inspecting them periodically and inspection other recombinant DNA facilities as deemed necessary; and

IV-E-4-c. Announcing and distributing certified HV2 and HV3 host-vector systems (see Section II-E-3).

(See Administrative Practices Supplement for additional information on the administrative procedures of ORDA and other NIH components.)

IV-F. *Registration*

IV-F-1. *Required Registration.* Institutions receiving NIH funds for recombinant DNA projects shall inform NIH of all recombinant DNA projects at the institution. A non-NIH project, after approval by the Institutional Biosafety Committee, shall be registered with NIH within 30 days of initiation. Applications for NIH projects must be accompanied by a Memorandum of Understanding and Agreement (MUA).

For information on MUAs or equivalent documents that must be submitted for registration of recombinant DNA projects, see the Administrative Practices Supplement (APS).

IV-F-2. *Federal Agency Registration.* Institutions at which recombinant DNA research projects funded by other Federal agencies are conducted need not register such projects with NIH when the Federal agency maintains a registry and provides such information to NIH. Registration of non-NIH-funded research with the NIH Office of Recombinant DNA Activities (ORDA) is described in the APS. (The information required is similar to that in an MUA for NIH-supported research.)

IV-F-3. *Voluntary Registration and Certification.* Any institution that is not required to comply with the Guidelines may nevertheless register recombinant DNA research projects with NIH by submitting the appropriate information to ORDA. NIH will accept requests for certification of host-vector systems proposed by the institition. The submitter must agree to abide by the physical and biological containment standards of the NIH Guidelines.

IV-F-4. *Disclosure of Information.* Institutions are reminded that they should consider applying for a patent before submitting information to DHEW which they regard as potentially proprietary. (Provisions for protection of proprietary information as permitted under current DHEW authorities will be proposed as a future supplement to these Guidelines.)

IV-G. *Compliance.* As a condition for NIH funding of recombinant DNA research, Instititions must ensure that such research conducted at or sponsored by the Instititon, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV-G-1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV-G-2. All non-NIH-funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV-G-3. Information concerning noncompliance with the Guidelines may bc brought forward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution, generally through the IBC, shall take appropriate action. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV-G-4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHEW and Public Health Service procedures shall govern.

V. FOOTNOTES AND REFERENCES

1. The reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. The list of organisms in each class, as given in this publication, is reprinted in Appendix B to these Guidelines.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as Class 2 in the *Classification of Etiologic Agents on the Basis of Hazard*, 4th Edition, July 1974, as Class 1 agents for the purposes of these Guidelines (see Section IV-E-1-b-(2)-(d)). An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20014.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

One exception to the prohibition of formation of recombinant DNAs derived from Class 3, 4, or 5 agents is that the formation of recombinant DNAs derived from Vesicular Stomatitis Virus (VSV) is not prohibited. The reason for this is explained in the "Decision Document" accompanying the proposed revised guidelines published in the FEDERAL REGISTER on July 28, 1978. However, as noted in Appendix B, a permit from the U.S. Department of Agriculture is required for the import or interstate transport of VSV. This can be obtained form USDA-APHIS, Veterinary Service, Federal Building, Hyattsville, Maryland 20782.

2A. In Parts I and III of the Guidelines, there are a number of places where judgments are to be made. These include: "cells known to be infected with such agents" (Section I-D-1); "toxins potent for vertebrates" (Section I-D-2); "beyond that which occurs by natural genetic exchange" (Section I-D-3); "known to acquire it naturally" (Section I-D-5); "known to produce a potent polypeptide toxin * * * or known to carry such pathogens * * * not likely to be a product of closely linked eukaryote genes * * * shown not to contain such agents" (Section III-A-1-a-(5)-(a)); "shown to be free of disease causing microorganisms" (Section III-A-1-a-(5)-(b)); "close relatives" (Section III-C-3); and "produce a potent polypeptide toxin" (Footnote 34).

In all these cases the principal investigator is to make the initial judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV-D-7-a). In all these cases, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its rsponsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV-D-3-a-(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV-E-3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interesting and determining containment levels upon request by ORDA" (Section IV-E-1-b-(2)-(a)).

3. The following types of data should be considered in determining whether DNA recombinants are "characterized" and the absence of harmful sequences has been established: (a) The absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or the host or be detrimental to humans, animals, or plants); (b) the types(s) of genetic information on the cloned segment and the nature of transcriptional and translation gene products specified; (c) the relationship

between the recovered and desired segment (e.g., hybridization and restriction endonuclease fragmentation analysis where applicable); (d) the genetic stability of the cloned fragment; and (e) any alterations in the biological properties of the vector and host.

4. In Section I-E, "exemptions" from the Guidelines are discussed. Such experiments are not covered by the Guidelines and need not be registered with NIH. In Section I-D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that an experiment may be expressly released from a prohibition. At that time it will be assigned appropriate

5. Care should be taken to inactivate recombinant DNA before disposal. Procedures for inactivating DNA can be found in the "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research."

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20. Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *Class I* is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) With a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

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34. We are specifically concerned with the remote possibility that potent toxins could be produced by acquiring a single gene or cluster of genes. See also footnote 2A.

35. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I-E-4.

36. As classified in the Second Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, Frank Fenner, Ed. Intervirology 7 (19-115) 1976. (As noted in the Prohibition Section, the use of viruses classified[1] as Class 3, 4, or 5, other than VSV, is prohibited.)

37. The cDNA copy of the viral mRNA must be >99% pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

37A. For the purpose of these Guidelines, viruses of the families *Papovaviridae, Adenoviridae,* and *Herpetoviridae* (36) should be considered as "transforming" viruses. While only certain of these viruses have been associated with cell transformation *in vivo* or *in vitro,* it seems prudent to consider all members to be potentially capable of transformation. In addition, those viruses of the family *Poxviridae* that produce proliferative responses—i.e., myxoma, rabbit and squirrel fibroma, and Yaba viruses—should be considered as "transforming."

38. >99% pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.

39. The viruses have been classified by NCI as "moderate-risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recommendations on handling the viruses themselves.

40. EK1CV means the use of an EK1 host and a vector certified for use in an EK2 system.

41. The DNA preparation is defined as "purified" if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

42. The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

43. This is not permitted, of course, if it falls under any of the Prohibitions of Section I-D. Of particular concern here is prohibition I-D-5, i.e., "Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture."

44. Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. It should be pointed out that risk of laboratory-acquired infection as a consequence of tissue culture manipulation is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50-100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, there should be a clear idea of the location within the molecule of:

(i) The sites at which DNA synthesis originates and terminates,

(ii) The sites that are cleaved by restriction endonucleases, and

(iii) The template regions for the major gene product.

If possible the helper virus genome should: (i) Be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) Consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

45. Review by NIH on a case-by-case basis means that NIH must review and set appropriate containment conditions before the work may be undertaken. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin.*

46. Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

47. >99% pure; otherwise as for shotgun experiments.

APPENDIX A

Section I-E-4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I-E-4 of these revised Guidelines are recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms of the following classes, and (2) to be propagated in any of the organisms listed below. (Classification of *Bergey's Manual of Determinative Bacteriology,* eighth edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

1. Genus *Escherichia*
2. Genus *Shigella*
3. Genus *Salmonella* (including *Arizona*)
4. Genus *Enterobacter*
5. Genus *Citrobacter* (including *Levinea*)
6. Genus *Klebsiella*
7. *Erwinia amylovora*
8. *Pseudomonas aeruginosa*
9. *Serratia marcescens*

APPENDIX B

CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

I. Classification of Etiologic Agents on the Basis of Hazard (1)

A. *Class 1 Agents*

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

B. *Class 2 Agents*

1. *Bacterial Agents*

Actinobacillus—all species except. *A. mallei,* which is in Class 3
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Diplococcus (Streptococcus) pneumoniae
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellae vaginicola
Klebsiella—all species and all serotypes
Leptospira interrogans—all serotypes
Listeria—all species
Mima polymorpha
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae,* which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
Pasteurella—all species except those listed in Class 3
Salmonella—all species and all serotypes
Shigella—all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

2. *Fungal Agents*

[1] *Actinomycetes* (including *Nocardia* species and *Actinomyces species and Arachnia propionica*)
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

3. *Parasitic Agents*

Endamoeba histolytica
Leishmania sp.
Waegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

4. *Viral, Rickettsial, and Chlamydial Agents*

Adenoviruses—human—all types
Cache Valley virus

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[1] Since the publication of the classification in 1974 [1], the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Corona viruses
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except *Parainfluenza virus 3, SF4 strain*, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox, Monkey pox, and Whitepox*, which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus*, which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus*, which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. *Class 3 Agents*

1. *Bacterial Agents*

Actinobacillus mallei [2]
Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains [2])
Pseudomonas pseudomallei [2]
Yersenia pestis

2. *Fungal Agents*

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. *Parasitic Agents*

Schistosoma mansoni

4. *Viral, Rickettsial, and Chlamydial Agents*

Alastrim, Smallpox, Monkey pox, and Whitepox, when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboriruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)

[2] USDA permit also required for import or interstate transport.

Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Vesicular stomatitis virus [2]
Yellow fever virus—wild, when used *in vitro*

D. *Class 4 Agents*

1. *Bacterial Agents*

None

2. *Fungal Agents*

None

3. *Parasitic Agents*

None

4. Viral, Rickettsial, and Chlamydial Agents

Alastrim, Smallpox, Monkey pox, and Whitepox, when used for transmission or animal inoculation experiments
Hemorrhagic fever agents, including *Crimean hemorrhagic fever (Congo), Junin*, and *Machupo* viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever*, and *Central European encephalitis viruses*
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used for transmission or animal inoculation experiments

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (2)

A. *Low-Risk Oncogenic Viruses*

Rous Sarcoma	Rat Leukemia
SV-40	Hamster Leukemia
CELO	Bovine Leukemia
Ad7-SV40	Dog Sarcoma
Polyoma	Mason-Pfizer Monkey Virus
Bovine papilloma	Marek's
Rat mammary tumor	Guinea Pig Herpes
Avian Leukosis	Lucké (Frog)
Murine Leukemia	Adenovirus
Murine Sarcoma	Shope Fibroma
Mouse mammary tumor	Shope Papilloma

B. *Moderate-Risk Oncogenic Viruses*

Ad2-SV40	GaLV
FeLV	HV ateles
HV Saimiri	Yaba
EBV	FeSV
SSV-1	

III. Animal Pathogens (3)

A. *Animal disease organisms which are forbidden entry into the United States by Law (CDC Class 5 agent)*

1. Foot and mouth disease virus

B. *Animal disease organisms and vectors which are forbidden entry into the United States by USDA Policy (CDC Class 5 Agents)*

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exantema virus
Wesselsbron disease virus
Zyonema farciminosum (pseudofarcy)

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Dated: December 15, 1978.

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Approved: December 15, 1978.

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Approved: December 15, 1978.

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